

Department of Parasitology, Polish Academy of Sciences
Head: Prof. Dr. Witold Stefański
Department of Pharmacology, Agricultural College of Warszawa
Head: Prof. Dr. Michał Gedroyć

I. POCZTARSKA-WĘGRZYN, Z. PRZYJAŁKOWSKI,
M. RUTKOWSKA

Effect of pharmacological hibernation on the development of
Trypanosoma equiperdum (Doflein 1901) in rats

Wpływ hibernacji farmakologicznej na rozwój *Trypanosoma equiperdum*
(Doflein 1901) u szczurów

First observations on the inhibited development of *Trypanosoma equiperdum* Doflein 1901 during hibernation of animals were reported by Blanchard 1903, Brumpt 1908, and Branden 1939, who confirmed the inhibition of development of *Trypanosoma* during the hibernation of *Eliomys quercinus* L.

It may be assumed that in many animals the development of various parasites, and among them even worms, is inhibited during hibernation. Artificial hibernation, in contradistinction to a natural one, can be induced by means of drugs decreasing metabolism. Though artificial hibernation is not an equivalent of a natural one, the effect that the former may have on the inhibition of development of parasites was a matter of interest to biologists and clinicians.

The following drugs were used in order to induce artificial hibernation: several mixtures such as the so-called lytic cocktails, including local anesthetics, ganglioplegics, parasympatheticolytic and sympatheticolytic drugs, cortex and stem paralyzants, or narcotica. All these drugs cause the decrease of metabolism. Besides, the ambient temperature was lowered to induce hypothermia in the experimental animals with the result of inhibiting metabolism still more efficiently.

Floowing their own investigation on the hibernation of rats, the present authors familiarized themselves with the paper of Bouisset, Harrant et Ruffie 1956, who undertook investigations on the same subject in similar material.

The French authors used a lytic cocktail containing: Largactil 0.025 g per 5 ml (1 volume), Dolosal 0.1 g per 2 ml (0.5 volume), and Phenergan 0.05 g per 5 ml (0.5 volume). They carried out the experiments on 25 white rats (*Rattus norvegicus albino*), the internal temperature of which was 37°C. The rats were infected with 80 — 240 million of *Trypanosoma* each. Single 0.5 ml dose of hibernating mixture had been given to the rats, and this resulted in a decrease of the temperature to 30°C. When the temperature rose to 32°C, 0.2 ml of the mixture was again given to the rats. The reduction of the internal temperature to less than 30°C caused death of animals due to heart shock.

The French authors divided the experimental rats into a group to be hibernated, and the control one, to which no lytic mixture was given. In both groups, trypanosomes appeared already 36 hours after infection. Out of 10 controls, 6 animals lived for 60 hrs., 3 — 72 hrs., and 1 — as long as 84 hrs. Rats hibernated immediately after infection lived for 84 hrs., or even 96 hrs. The increase of the number of trypanosomes, though steady in both groups, was slower in hibernated rats than in controls.

An attempt was made in our experiments to create conditions which would be more or less similar to those occurring in natural hibernation, i.e. the metabolism was decreased and the body temperature was lowered to the possible limit. This was maintained for several days to find the differences in the speed of increase of trypanosomes as in hibernated and control animals.

In our experiments, the heart shock occurred at a temperature below 25°C, and was probably due to narcotica such as luminal which paralyzes the spinal cord and neural centres.

Material and methods

Experiments were carried out with white rats (*Rattus norvegicus* Berkenhout 1769), the weight of which varied between 200 and 300 g.

Out of 126 rats used, nearly half died 24 hrs. or 48 hrs. after infection, most probably as a result of the poisonous effect of the mixture. After drugs had been given to certain population, some animals were found to be more and some less sensitive to a particular drug.

The remaining 64 rats were divided into 6 groups, the total number of the animals to be hibernated being 40 and of the controls — 24.

Laborit et Huguenard 1956 method of preparing hibernating mixture was used: 3 ml of solution containing 75 mg of Largactil, 2.4 ml of solution containing 24 mg of Dolantine, and 2 ml of solution containing 50 mg of Phenergan.

In order to induce paresis of spinal cord luminal was administered to the animals, as is done by clinicians in hibernating people. The dose used was 220 mg of luminal-natrium in 8 ml of distilled water.

The blood for calculation of the consecutive numbers of *Trypanosoma* was taken from the tail every 24 hrs., starting from 72 hrs. after infection. It was collected into a Peten shaker to the 0.5 scale and diluted 1:200 with Hayem liquid. The number of *Trypanosoma* was calculated twice in Thomas-Zeiss chamber, in 5 large squares (diagonally + the edge square), and the average found. The number of *Trypanosoma* in 1 cmm of blood was calculated by the simplified formula for erythrocytes, i.e., the average number of *Trypanosoma* in 10 large squares was multiplied 10,000 times.

Experiments

The temperature of rats used for the experiments varied between 30° and 33°C, and sometimes was even 35°C. All the animals were injected peritoneally with 100 to 6 million of *Trypanosoma equiperdum*. 5 — 7 rats of each series were hibernated, while the remaining ones were controls.

The group to be hibernated was injected intramuscularly with 0.2 hibernating mixture 3 times a day (at 10 a. m., 3 p. m., and 7 p. m.). In order to induce anesthesia of the spinal chord the animals were injected intramuscularly with 0.1 ml of luminal twice a day (at 10 a. m., and 7 p. m.). Before

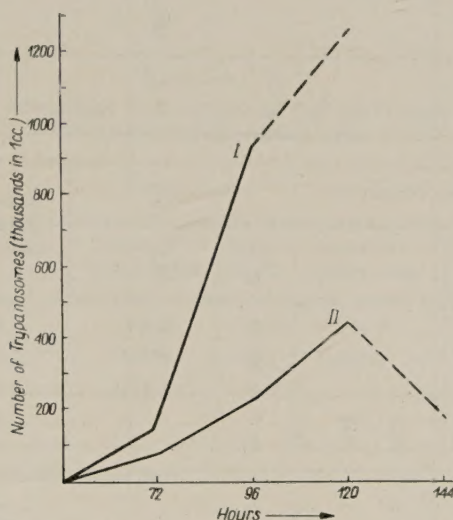


Diagram I. Multiplication of *T. equiperdum* of the blood of hibernated and control rats. I — control rats, data for one series; II — hibernated rats, data for two series

each administration of the mixture the temperature of the rats was taken, and when it attained 25°C the administration of both luminal and mixture was discontinued. After an increase of the temperature above 25°C the hibernation mixture and luminal were administered again.

On the third day, i. e., 72 hrs. after infection the blood was taken from each rat and the number of *Trypanosoma* in 1 cmm of blood computed.

Results and discussion

As a result of the administration of the hibernating mixture and narcotic, as well as of the cooling of ambient temperature down to + 4°C, the temperature of the rats dropped to an average of 26°C, or even to 16.5°, 18.5°, or 21°C in some rats which, however, died after the temperature attained this point.

Consequent upon pharmacological hibernation was a decrease in the average number of *Trypanosoma* in each series of the experimental rats as compared to the controls (Table II). The hibernation, however, during the full increment of *Trypanosoma*, failed to inhibit the development of this parasite.

T a b l e I
Development of *T. equiperdum* in the blood of hibernated and control rats

Animals	Series	Dose of trypanosomes	Number of parasites (10^3 /cmm of blood) after hours:											
			72		96		106		120		144		168	
			No.	°C	No.	°C	No.	°C	No.	°C	No.	°C	No.	°C
Hibernated	I	6000000	30.0	29.4										
	II	500000	115.0*		65.0	29.7			550.0*	28.0				
	III	475000	0		153.3	28.5			987.5	22.2				
	IV	200000	0		50.0*	27.5			222.5	30.0				
	V	500	67.9	23.2	0	20.0	1255.0	19.5	77.5	29.7	367.5	23.2		
	VI	100	0		0		0		0		28.7	29.5	577.5	27.2
Controls	I	6000000	50.0		940.0									
	II	500000	297.5		1510.0									
	III	475000	0		0		0		583.3					
	IV	200000	0		736.6									
	V	500	80.9		533.3		1200.0*							
	VI	100	0		0		0		0		865.0*		783.3	385.0*
														650.0*

* data for one specimen in a series

It was generally found that in both hibernated and control group the period of time between the infection and the appearance of *Trypanosoma* averaged 72 hrs. at the least (Table I) and 168 hrs. at the most (series VI). Some series (IV and II) in the control group has, however, revealed an earlier appearance of trypanosomes than the hibernated one, the difference being 24 hrs.

Table II
Average development of *T. equiperdum* ($10^3/\text{cmm}$) in the blood
of hibernated and control rats

Time after injection (in hours)	Control rats		Hibernated rats	
	Number of trypanosomes	Temp. in °C	Number of trypanosomes	Temp. in °C
72	142.8	30.0	70.9	26.3
96	929.9	31.5	230.2	26.4
106	1255.0 *	30.0		
120			459.3	27.5

* data for one series only

In four experimental series, less trypanosomes were found in the blood of the hibernated animals as from the third day after infection, than in the controls. Thus, the control group in series I showed after 96 hrs. 940,000 *Trypanosoma* in 1 cmm of blood and the hibernated group only 65,000. In series II, 1,510,000 trypanosomes in 1 cmm of blood of controls were found after 96 hrs., and 153,000 in the respective hibernated group. In series V, however, the hibernated group showed 652,000/cmm after 96 hrs. and the controls no more than 633,000/cmm.

In series VI, infected with 100 trypanosomes, the parasites appeared, however, in the control group only after 168 hrs., the exception was one rat which showed the parasite after 144 hrs. In the respective control group, *Trypanosoma* occurred earlier, i. e., their number averaged 28,700 in 1 cmm of blood in 144 hrs. The control group of the series showed 783,300 parasites in 1 cmm while 577,500 specimens were found in the hibernated animals (Table I).

The average results from 5 series of both control and hibernated animals are given in Table II; the table indicates in control rats a steady increase of *Trypanosoma* till the death of animals, which occurred 96 — 106 hrs. after infection.

The average number of trypanosomes in the blood of hibernated animals has also increased, but the increase was considerably slower. No such regularity was observed in particular cases. The increment of trypanosomes in both groups is illustrated by the curve included.

Series VI was excluded from the average results since no such regularity has been observed as was in the remaining series.

Conclusions

Sensitivity of rats to the hibernating mixture administered with narcotic drugs varies on a wide scale.

The development of *Trypanosoma* in all five groups has been inhibited by pharmacological hibernation. One series (VI) has, however, shown a sudden increase of parasites.

In general, the comparison of the average results in hibernated and control groups has revealed a regular decrease of the number of parasites, and a later appearance of trypanosomes (24 to 48 hrs. later than in the controls) in hibernated rats which moreover survived longer than the controls. However, no such regularity was observed in particular cases.

It may be concluded that the artificial hibernation causes a decrease of metabolism in both the host and the parasite which consequently becomes less proliferative. This weaker proliferation of trypanosomes may be, however, due not only to temperature drop, but also to the toxicity of the drugs.

In the light of our experiments, the temperature of 30°C does not seem to be low enough to inhibit the development of the parasites.

Authors' address:
Zakład Parazytologii P. A. N.
Warszawa, Pasteura 3

LITERATURE

1. Blanchard R. — Experiences et observations sur la Marmotte en hibernation. Réceptivité à l'égard des Trypanosomes. C. R. Soc. Biol., 64, 1903.
2. Bouisset L., Harant H., Ruffie J. — Hibernation et Parasitisme. Montpellier Medical, 49, 1956.
3. Bouisset L., Harant H., Ruffie J. — Action de l'hibernation expérimental sur l'évolution de *Trypanosoma equiperdum* (Doflein 1901) chez le Rat blanc. C. R. Soc. Biol., 150, 1956.
4. Bouisset L., Harant H., Ruffie J. — Parasitose expérimentale a *Trypanosoma equiperdum* Doflein. Ann. Parasitol., 31, 1956.
5. Branden F. von — Influence du froid sur l'évolution de la trypanosomiase du rat blanc infecté de *Trypanosoma brucei*. Ann. Soc. Belg. Med. Trop., 243, 1939.
6. Brumpt E. — Guérison de la maladie du sommeil chez le Léroto vulgaire en hibernation. Action du froid sur le *Trypanosoma inopinatum*. C. R. Soc. Biol., 64, 1908.
7. Laborit H., Huguenard P. — Etude expérimentale du conditionnement ionique aux basses températures. C. R. Soc. Biol., 150, 1956.

STRESZCZENIE

Autorzy stwierdzili duże wahania wrażliwości szczurów na mieszanke hibernizującą podawaną razem ze środkiem narkotycznym, jak to jest stosowane w celu pewniejszego obniżenia przemiany materii (patrz metoda Laborit'a).

Uzyskano średnie obniżenie temperatury wewnętrznej szczurów z 33°C do 26°C, a w pewnych przypadkach nawet do 16,5, 18,5 lub 21°C. W naszych badaniach świdorowce pojawiały się we krwi szczurów najwcześniej po 72 godz.

Na 6 serii szczurów, w 5 seriach u grup hibernizowanych wystąpiło: 1) hamowanie namnażania świdorowców, 2) przedłużenie okresu przeżywania szczurów, oraz niekiedy 3) późniejsze nagłe pojawianie się świdorowców we krwi szczurów (o 24 — 48 godz. w porównaniu z grupą kontrolną). Jednakże w indywidualnych przypadkach regularności tych zjawisk nie da się ustalić.

Na podstawie naszych doświadczeń doszliśmy do wniosku, że obniżenie temperatury ciała szczurów do 30°C nie jest, na ogół, przyczyną wyraźnego hamowania rozwoju pasożytów. Sądzymy, że w hamowaniu rozwoju pasożytów dużą rolę odgrywa również działanie środków farmakologicznych.

Problem ten można rozstrzygnąć przez porównanie badań nad hibernacją farmakologiczną i hibernacją naturalną.

